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THE MOLECULAR WEIGHT OF RIBOSOMAL RIBONUCLEIC ACIDS AMONG THE PROTOSTOMIA

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Abstract

The molecular weights of the two larger rRNAs (18S and 26S) of one deuterostome and five protostomes were determined from their electrophoretic mobility. The molecular weight of the smaller (18S) ribosomal RNA was the same in all groups tested, but the molecular weight of the larger ribosomal RNA (26S) was 14 per cent smaller among the protostomes than the corresponding RNA (28S) of the deuterostomes.

Introduction

The size of ribosomal RNAs are moderately constant among the eucaryotes; however, the rRNA of the large subunit (the nominal 28S rRNA) is larger among mammals and birds and smaller among lower chordates and invertebrates (Perry *et al.*, 1970; Lewin, 1980). This pattern is well supported by analytical data for chordates and echinoderms, but there is a paucity of data among the Protostomia.

I report measurements of the molecular weights of the two larger rRNAs from five protostomes, a siliceous sponge (*Microciona prolifera*), an anemone (*Eloactis producta*), a nemertine worm (*Cerebratulus lacteus*), a mussel (*Mytilus edulis*), a clam (*Spisula solidissima*), and one deuterostome, the asteroid blood star (*Henricia sanguinolenta*). Observations showing the heat lability of the 28S rRNA of these invertebrates are also reported.

Materials and Methods

RNA extraction

Animals were obtained from the Supply Department of the Marine Biological Laboratory, Woods Hole, MA. Either the entire specimen, or, for molluscs and asteroids, soft tissues, exclusive of the gut, were removed and homogenized at 4°C in a high-shear mixer with 5 ml of homogenizing solution (1% triisopropylnapthylenedisulfonate, 1% sodium dodecyl sulfate, 0.1% Macaloid, 10^{-2} *M* magnesium acetate, 10 μg/ml polyvinylsulfonate in 0.01 *M* sodium acetate, pH 5.0) and 5 ml of phenol per g of tissue. The homogenate was centrifuged, the aqueous phase recovered, and extracted again with phenol. The final aqueous phase was recovered by centrifugation and precipitated by the addition of NaCl to 0.1 *M* and 2 volumes of 95% ethanol and storage at −20°C overnight. RNA for molecular weight standards was extracted from rat liver and *E. coli* by the same procedure.

The heat lability of rRNA was determined by heating a sample of RNA from each specimen for 5 min at 60°C. After electrophoresis, as described below, the area under the gel scan was measured and the per cent of the 28S rRNA that degraded was calculated by comparison with an unheated preparation of rRNA.

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Electrophoresis

The precipitated RNA was dissolved in 0.04 *M* tris-acetate buffer, pH 7.4, and layered over a 2.4 per cent acrylamide gel contained in a 6 × 0.5 cm glass tube

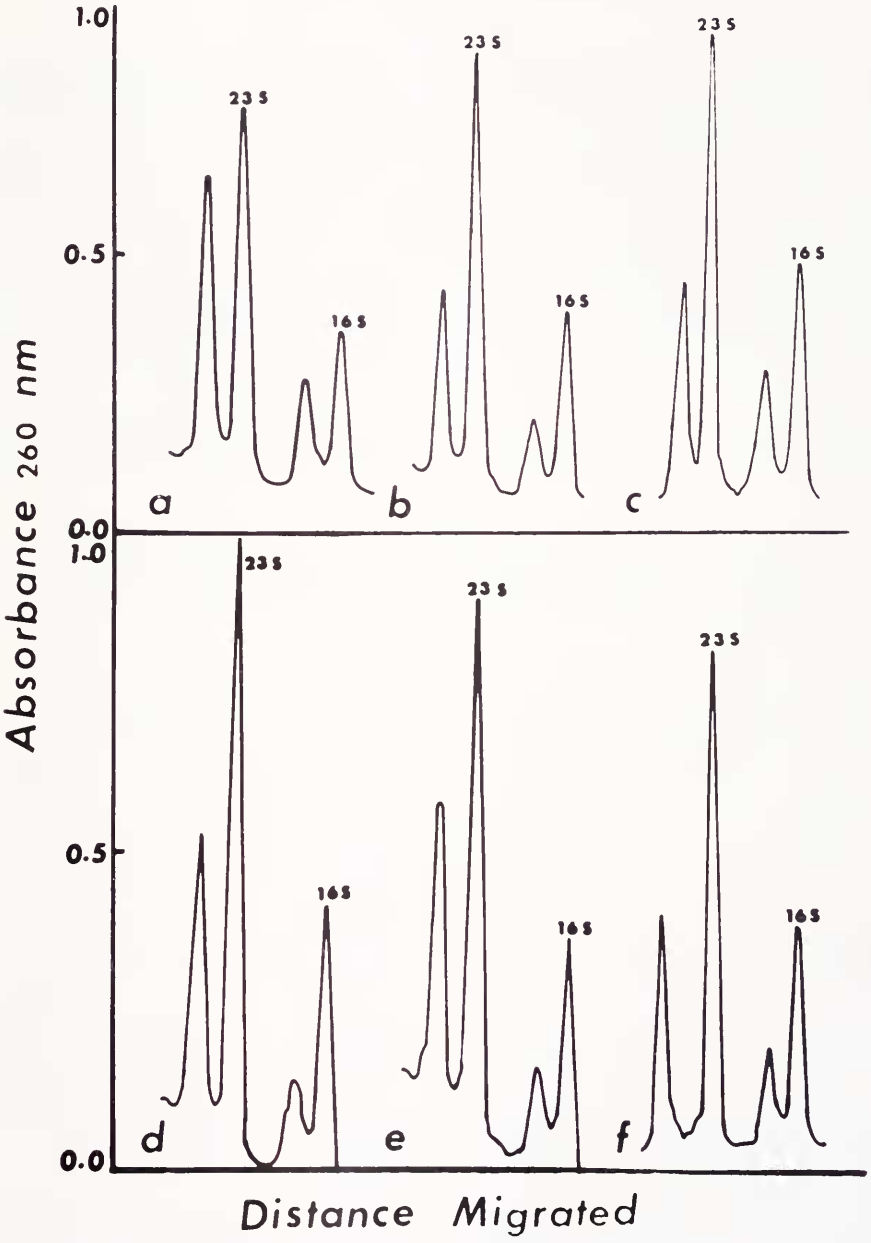

FIGURE 1. Relative mobility of RNA samples and RNA standards. The peaks marked 16S and 23S are RNA standards from *E. coli*. Ribosomal RNAs are from: (a) *Microciona prolifera*, (b) *Eloactis producta*, (c) *Cerebratulus lacteus*, (d) *Spisula soldissima*, (e) *Mytilus edulis*, and (f) *Henricia sanguinolenta*.

(Loening, 1968). Electrophoresis was at a constant current for 5 mA per gel for 45–60 min in a buffer of 0.04 *M* tris-acetate, pH 7.4, containing 0.2% sodium dodecyl sulfate. After electrophoresis the gels were placed in a quartz cuvette and scanned at 260 nm in a Gilford spectrophotometer.

Results

RNA standards were co-run with each experimental sample and the relative mobility of each sample and its set of standards was plotted against the log molecular weight of the standards. Relative mobilities were determined by measuring the distance each species of RNA migrated into the gel (Fig. 1). The molecular weights of the unknown RNAs were calculated from the linear relation between molecular weight and relative mobility of the RNA standards. That these RNAs were ribosomal RNA was evident from their size and abundance.

Table I contains replicate measures, average molecular weights, and standard errors for the two larger rRNAs of each of the six species studied. Among these were one deuterostome, a starfish, and five protostomes (two molluscs, a nemertine worm, an anemone, and a sponge). Also in Table I, column 4, are the per cent degradation of the 28S rRNA caused by heating at 60°C for 5 min. The 18S rRNAs of all species were stable when heated at 60°C for 5 min. It is not clear why the 26S rRNA of *Mytilus, Spisula,* and *Cerebratulus* were completely degraded by heating at 60°C (Table I).

Discussion

The molecular weight of 18S rRNA does not vary significantly among the species listed in Table I. The size of this RNA has also been found to be constant among

Table I

Molecular weight and thermal stability of ribosomal RNAs

Organism	Molecular weight × 10^{-6} Large rRNA		Small rRNA		% degradation of 26 S RNA
Henricia sanguinolenta	1.56 1.59 1.61	1.59 ± 0.015*	0.70 0.71 0.69	0.70 ± 0.006	16
Spisula soldissima	1.37 1.38 1.40	1.38 ± 0.009	0.70 0.70 0.71	0.70 ± 0.003	100
Mytilus edulis	1.40 1.40 1.38	1.39 ± 0.007	0.69 0.69 0.67	0.68 ± 0.007	100
Cerebratulus lacteus	1.32 1.33 1.33	1.33 ± 0.003	0.71 0.71 0.72	0.72 ± 0.003	100
Eloactis producta	1.38 1.37 1.38	1.38 ± 0.003	0.70 0.70 0.71	0.70 ± 0.003	21
Microciona prolifera	1.36 1.37 1.37	1.37 ± 0.003	0.71 0.71 0.72	0.71 ± 0.003	11

* Mean and standard error of mean.

other organisms in which it has been measured (Table II). In contrast to this consistency in the size of 18S rRNA throughout the animal kingdom, the molecular weight of the larger rRNA of the protostomes is 14 per cent smaller than the corresponding rRNA of the single deuterostome reported in Table I and of those deuterostomes previously studied (Loening, 1968; Attardi and Amaldi, 1970; Perry *et al.*, 1970; and Lewin, 1980). However, the size of the larger rRNA is invariant among the protostomes observed and among representatives of two groups, sponges and coelenterates, of their immediate phylogenetic predecessors. Therefore, it appears that a major increase in the size of the RNAs of the large ribosomal subunit occurred as the deuterostomes diverged from the protostomes, and that other changes in size did not arise among the older protostomes or their immediate predecessors.

Among the protozoa, with the exception of *Acanthamoeba* (Loening, 1968), the larger RNA is 5.8% smaller than the average size of this RNA among the protostomes. (The size of the *Acanthamoeba* rRNA is uncertain as the RNA used for molecular weight determination was unstable (Loening, 1968).) While the protozoan rRNA may be smaller than the protostomian rRNA there is not enough data avail-

TABLE II

Molecular weights of invertebrate ribosomal RNAs

Organism	Molecular weight $\times 10^{-6}$		Reference
	Large rRNA	Small rRNA	
Protozoa			
Acanthamoeba	1.53	0.89	Loening (1968)
Euglena gracilis	1.30	0.85	Rawson and Stutz (1968)
Tetrahymena pyriformis	1.30	0.69	Loening (1968) Kumar (1969)
Paramecium	1.31	0.69	Loening (1968) Reisner *et al.* (1968)
Porifera			
Microciona prolifera	1.37	0.71	Table I
Coelenterata			
Eloactis producta	1.38	0.70	Table I
Nemertina			
Cerebratulus	1.33	0.72	Table I
Nematoda			
Ascaris lumbricodes	1.42	0.76	Tobler *et al.* (1974)
Arthropoda			
Drosophila	1.40	0.73	Loening (1968)
	1.40	0.68	Hastings and Kirby (1966)
	1.40	0.65	Perry *et al.* (1970)
Mollusca			
Spisula solidissima	1.38	0.70	Table I
Mytilus edulis	1.39	0.68	Table I
Mulinia lateralis	1.34	0.75	Kidder (1976)
Ilyanassa obsoleta	1.37	0.70	Koser and Collier (1971)

able to establish that this difference is significant. Thus, the possibility remains that an increase in the size of the RNA of the large ribosomal subunit occurred before or at the time of the appearance of the sponges.

From estimates of sequence diversity of rRNA from a number of organisms Pinder *et al.* (1969) found that the structure, but not the exact sequence, of rRNA was conserved during evolution. These observations suggest that changes in nucleotide sequence of rRNA are tolerated as long as the overall size and shape of the rRNA is suitable for the assembly of a ribosome that will function efficiently in protein synthesis. Thus, the reduced size of the protostomian rRNA is suitable, as is the still smaller rRNA of procaryotes, for the construction of a satisfactory ribosome. It may be found, however, that the ribosomal proteins of protostomian ribosomes are different, as are the proteins of procaryotic ribosomes, from deuterostomian ribosomes.

The degradation of the 26S rRNA after heating at 60°C (Table I), as has been previously reported for a number of organisms (Koser and Collier, 1971; Shine and Dalgarno, 1973), indicates that one or more fragments of RNA are hydrogen-bonded to the main 26S rRNA component. The data reported here show, for the first time, that these rRNA fragments exist in a sponge and an anemone, which suggests that this organization of rRNA existed before the appearance of the protostomes.

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